AD_____

GRANT NUMBER DAMD17-94-J-4078

TITLE: Genomic Instability at Premalignant and Early Stages of Breast Cancer Development

PRINCIPAL INVESTIGATOR: C. Marcelo Aldaz, M.D.

CONTRACTING ORGANIZATION: The University of Texas
M.D. Anderson Cancer Center
Houston, Texas 77030

REPORT DATE: Annual

TYPE OF REPORT: August 1996

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	August 1996	Annual (1 Aug 95 - 31 Jul 96)

4. TITLE AND SUBTITLE
Genomic Instability at Premalignant and Early Stages of Breast Cancer Development

5. FUNDING NUMBERS
DAMD17-94-J-4078

6. AUTHOR(S)
C. Marcelo Aldaz, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of Texas M.D. Anderson Cancer Center
Houston, Texas 77030

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 2170-25012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200*

We have developed techniques that allow the analysis of multiple chromosomal loci from single paraffin sections from breast cancer lesions. This approach is being used to allelotype small preinvasive breast cancer lesions. We identified the chromosome arms most frequently affected by allelic losses and imbalances at preinvasive stages of breast carcinogenesis and those allelic losses involved in more advanced stages of progression. We have now performed a high resolution allelotype of chromosome 16q. This allowed us to refine the location of specific subchromosome regions containing putative tumor suppressor genes of relevance on early breast carcinogenesis. We also observed that microsatellite instability (replication error phenotype, RER^+) is characteristic of a subset of breast tumors (invasive lobular breast carcinomas), indicating the possible involvement of defective DNA mismatch repair genes in these tumors. Studies are ongoing to address this point and to evaluate the use of RER phenotype determination as a prognostic-diagnostic tool.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Breast Cancer	18
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

____ Where copyrighted material is quoted, permission has been obtained to use such material.

____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

__X__ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

__X__ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

__X__ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

8.28.96

PI - Signature Date

TABLE OF CONTENTS

I. Subtitle: High resolution allelotype of chromosome 16 in Ductal carcinoma *in situ* of the breast: Refining a tumor suppressor gene region.

I.a Introduction

Numerous studies have focused on the identification and analysis of specific gene mutations and chromosome abnormalities in sporadic cancer, but to date no clear model of the critical events or delineation of primary abnormalities has emerged (1). Ductal carcinoma *in situ* (DCIS) of the breast is known as a preinvasive stage of breast cancer and is probably the precursor of infiltrating breast cancer (2). Genetic alterations shown at this stage might indicate association with early events in malignancy or invasiveness. Loss of heterozigosity (LOH) at specific chromosomal loci has been considered as part of the indirect evidence for postulating the existence of possible tumor suppressor genes within those specific chromosome regions. It is known that several mechanisms can lead to the loss of alleles in tumors such as chromosomal deletions, monosomies, mitotic recombination, and unbalanced translocation (3). Hypothetically, the remaining allele of the tumor suppressor gene in question could be rendered inactive due to events at the gene level such as specific point mutations or other types of inactivating mutations. Usually, LOH at specific chromosome regions affects not only the putative tumor suppressor gene but also neighboring genes or genetic markers that are used as indicators to track down the minimum area of LOH.

In the previous report we described the development of a technical approach that allowed us to perform LOH analyses (allelotypes) from paraffin embedded tissue samples (4). The basic technique involves tissue microdissection and microsatellite length polymorphism analysis. Using this micromolecular approach we compared the allelotypes of *in situ* and invasive breast cancer lesions. We observed that specific chromosome arms are more frequently affected by allelic losses and imbalances at preinvasive stages of breast cancer. In particular allelic losses affecting chromosome 16q as well as 17p, 17q and 7 appear to be early genomic abnormalities since they were observed in a significant number of DCIS lesions (5).

Loss of heterozygosity on chromosome 16q has been widely reported in breast (6-8), prostate (9) hepatoblastoma (10) and Wilm's tumor (11) with various frequency. In particular two regions on chromosome 16q have been revealed with very high frequency of LOH in breast cancer, one maps to region 16q22.1 (8) and a second one to 16q24.2-qter (7).

In the study reported here (manuscript in preparation) we extended our previous observations (5) and we used PCR microsatellite length polymorphism analysis, and tissue microdissection of paraffin embedded tumor samples to generate a high resolution deletion map of chromosome 16q in breast DCIS. We were able to identify a region of approximately 2-3 Mb in size for the location of a putative tumor suppressor gene of possible relevance in the development of breast cancer.

I.b Materials and Methods

Tumor Samples
DCIS samples were obtained from paraffin embedded blocks from the archives of the Department of Pathology of The University of Texas M. D. Anderson Cancer Center. A total of 35 cases of tumors collected and diagnosed as pure breast DCIS by our collaborating pathologist were analyzed. We did not include any DCIS with infiltrating components.

Paraffin tissue microdissection
The basic technical approach has been described previously (4). Minor modifications were introduced to improve efficiency of microdissection. Briefly, one to three five to eight micron thick paraffin sections were stained and used for microdissection. Using companion H&E stained slides as reference first tumor cells were microdissected using a fine point surgical blade (No. 11) under an inverted microscope. The edges of tumor area and stroma were cleared of debris using the same blade and blown with a stream of compressed air. A new blade was then used to dissect normal tissue the same way.

DNA preparation
Samples were rehydrated and DNA was extracted by incubating in 200 µl Instagene chelex matrix solution (BioRad) containing 60 µg of proteinase K in a shaking incubator at 37°C overnight. After proteinase K digestion, samples were boiled for 10 min., vortexed, and centrifuged at >7,000 G for 5 minutes. 5 µl aliquots of the supernatant were used for PCR amplification.

PCR microsatellite analysis
Primers for highly polymorphic human microsatellite repeats were purchased from Research Genetics (Huntsville, AL), as listed in Table 1. Prior to PCR reactions, the forward primer was end labeled using T4 polynucleotide kinase (Promega) and [γ32P]ATP (NEN 6,000 Ci/mmol). PCR reactions were performed in a 20 µl reaction volume containing 150 µM each dNTP, 1 unit Taq polymerase and 1X Taq buffer (Promega), 1.5 mM MgCl2, 1 pmole labeled primer and 2.5 pmole unlabelled forward and reverse primers. A hot start procedure was used in which template and primers were denatured at 96°C for 5 min, after which the remaining reaction constituents are added for 35-40 cycles at 94°C for 40 sec.; 55°C for 30 sec and 72°C for 30 sec. and a final elongation step of 72°C for 5 min. Products were electrophoresed on a 7% polyacrylamide sequencing gel at 90 watts constant power for 2-3 hrs. Gels were dried at 65-70°C for 1-2 hrs and exposed to X-ray film from 4 hrs to overnight. If necessary for certain primer sets the amplification conditions were further optimized by adjusting the MgCl2 concentration in the reaction buffer.

The sample was considered to have partial loss of heterozygosity, or allelic imbalance, if the signal intensity of one allele was diminished by approximately one-half or more of its normal intensity (i.e., in normal tissue) in relation to the remaining allele. Complete loss of heterozygosity was defined as a decrease of 90% or more in the signal intensity of one allele reactive to the other.

Yac clones spanning the region of interest were also purchased from Research Genetics (Huntsville, AB).

Table I Chromosome 16 Loss of Heterozygosity in Ductal Carcinoma *In Situ* of the Breast

Locus	Cumulative Linkage Map Distance(cM)(1)	Cytogenetic Location	No. of Cases	Tumor with LOH/ Informative	% LOH
D16S407		p13.13	34	2/33	6
D16S420	47	p12.3	31	3/24	13
D16S285		q12.1	34	4/24	17
D16S261		q12.1	34	6/20	30
D16S390		q12.2	32	5/23	22
D16S533		q21	33	7/23	30
D16S400	89	q21	32	7/11	64
D16S503	88	q21	28	10/21	48
D16S398		q22.1	34	13/28	46
D16S421	92	q22.1	32	12/22	55
D16S512	98	q22.1	33	9/21	43
D16S260		q22.2	29	8/16	50
D16S395		q23.1-q23.2	35	14/26	54
D16S515	100	q22.3-23.1	33	13/23	57
D16S518	103	q23.1-24.2	33	20/26	77
D16S504	109	q24.1	32	15/20	75
D16S516	108	q24.1	30	14/21	67
D16S507	113	q23.2	35	7/19	37
D16S393		q24.1	32	10/23	43
D16S422	119	q24.2	29	10/21	48
D16S402	120	q24.2	32	14/19	74
D16S413	137	q24.3	35	10/27	37

I.c Results

Paraffin microdissection of ductal carcinoma *in situ*
In order to optimize the microdissection of tumor and normal samples the sections were stained prior to dissection. In this way exact areas of tumor and normal tissues could be dissected from the same slide. Figure 1 shows three foci of DCIS (case 59) before (A) and after (B) microdissection. For each slide separate blades were used to dissect tumor and normal samples and a stream of compressed air was used to clear away debris prior to dissecting the normal tissue. Using this approach, from a single slide we can generate relatively pure tumor and normal genomic DNA pools which can be served as templates for about 20-30 PCR reactions.

Allelic loss and deletion map on chromosome 16q
We analyzed a total of 35 DCIS of the breast. We analyzed for LOH a panel of 22 microsatellite markers as summarized in Table 1 and Figure 2. Thirty one of these samples (89%) showed LOH or allelic imbalance in at least one or more chromosome 16 loci. In Figure 2 we schematically display the chromosome 16 allelotype of the individual DCIS tumors. As can be observed approximately one third of the cases displayed large terminal deletions or recombinations involving most or the whole chromosome 16 q arm. The remaining two thirds of LOH appear to be the result of mostly interstitial deletions or more complex recombination events. By overlapping the LOH pattern of the various tumors we could identify that the region between markers D16S515 and D16S516 (box in Figure 2) was the most commonly affected area. Figure 3 shows representative autoradiographs demonstrating allelic losses in tumor-derived DNA between q21-q24. Clear patterns of allelic losses as shown were observed throughout the experiment.

In Table 1 we display the summary information of the twenty two loci studied. Loci are arranged in linear order according to their cumulative linkage map distance, (Genethon Linkage Map, March 1996). Three distinct regions were observed with a very high percentage (~70% or above) of allelic losses among informative DCIS samples. The main region extends approximately from 16q23.1-q24.1, which spans markers D16S515 with a 57% frequency of LOH, D16S518 with losses in 20 of 26 informative DCIS samples (77%), D16S504 with 75% and D16S516 with 67%. The two other areas of high LOH are 16q24.2 including the D16S402 locus with a 74% frequency (14 of 19 informative cases) and 16q21 including D16S400 with LOH in 7 of 11 informative DCIS samples (64%). Other loci in the p-arm and in the q-arm more proximal to the centromere showed a much lower frequency of involvement.

As indicated D16S518 was the most commonly affected locus in the chromosome 16 q arm. In order to estimate the approximate physical size of the region with the highest frequency of overlapping deletion we used a YAC contig reported to span the region of interest according to Hudson et al. (12). We confirmed the location of the microsatellite markers D16S518-D16S504 and D16S516 to YAC clones 933h2 and 972d3 (data not shown). This area appears to be approximately 2-3 Mb in size and it is very likely located within chromosome bands 16q23.3-q24.1.

Figure Legends

Figure 1. Representative microdissection of a DCIS sample from paraffin embedded tissue section. A. Before microdissection note the three central structures, B. after microdissection.

Figure 2. Schematic representation of Chromosome 16q allelotype in breast DCIS. Each number on the top of the figure represents each individual tumor.
Black squares: LOH, Grey squares: Allelic imbalance, Open squares: no LOH. NI: non-informative locus.

Figure 3. Representative autoradiographs demonstrating LOH and allelic imbalance at various chromosome 16q loci as indicated. N: normal tissue, T: tumor (DCIS) tissue.

Figure 1

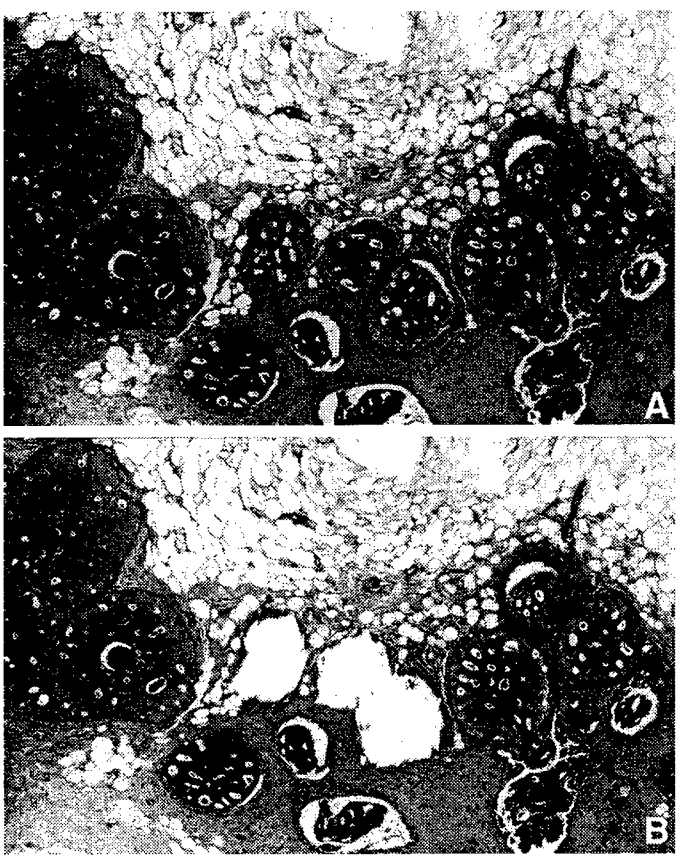


Figure 2

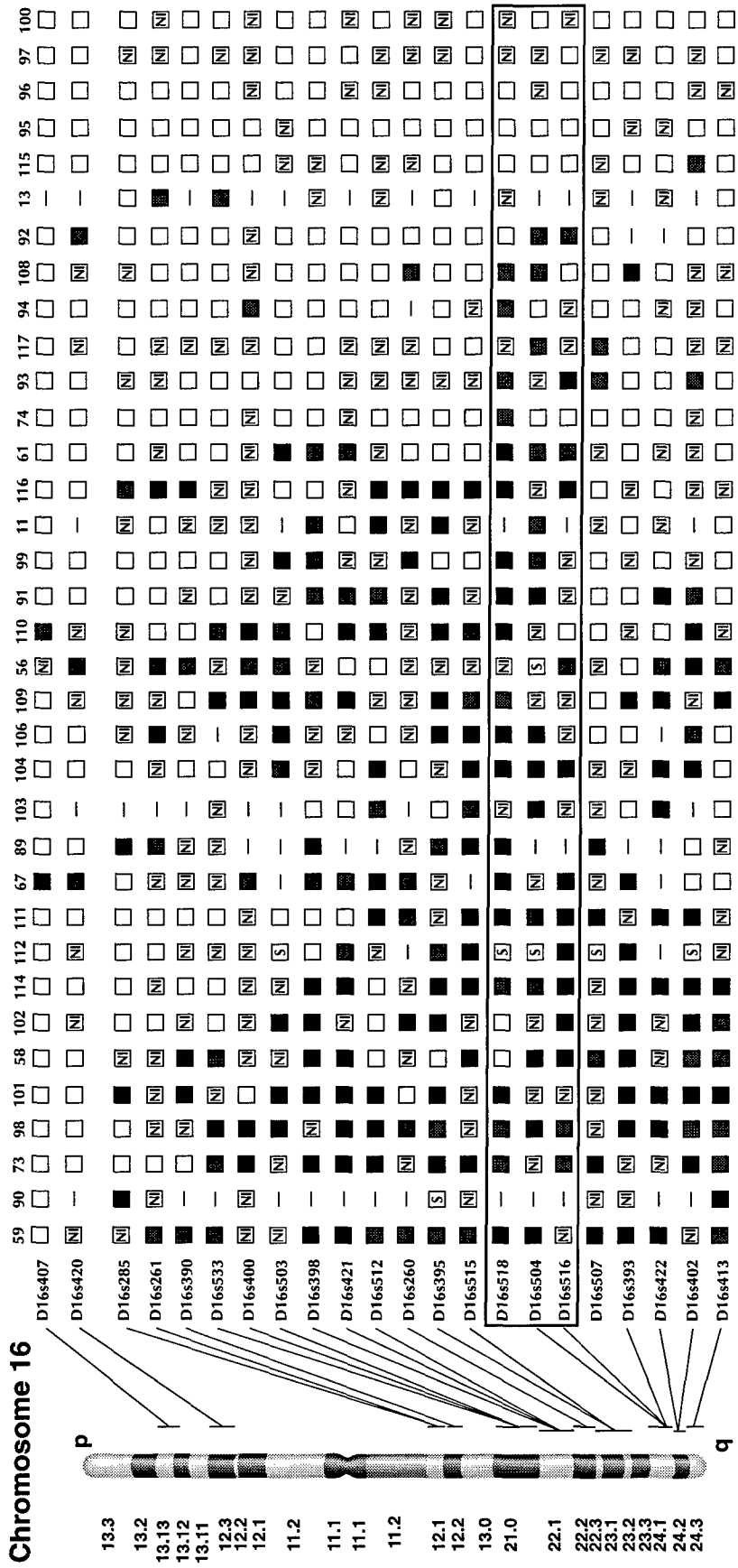
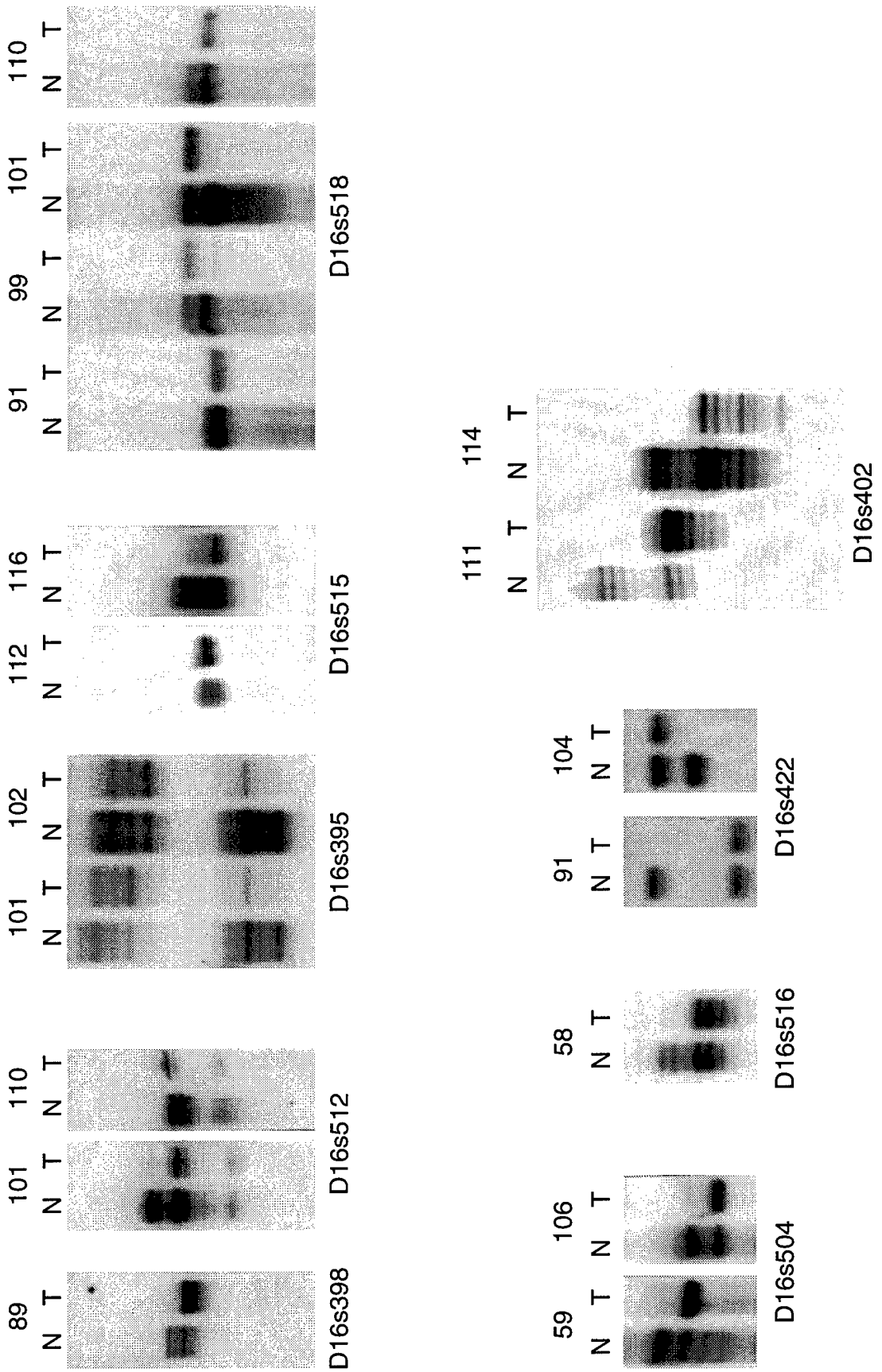

Chromosome 16
p
q
D16s407
D16s420
D16s285
D16s261
D16s390
D16s533
D16s400
D16s503
D16s398
D16s421
D16s512
D16s260
D16s395
D16s515
D16s518
D16s504
D16s516
D16s507
D16s393
D16s422
D16s402
D16s413
13.3
13.2
13.13
13.12
13.11
12.3
12.2
12.1
11.2
11.1
11.1
11.2
12.1
12.2
13.0
21.0
22.1
22.2
22.3
23.1
23.2
23.3
24.1
24.2
24.3

Figure 3


110
N T
D16s518
101
N T
99
N T
91
N T
116
N T
D16s515
112
N T
102
N T
D16s395
101
N T
110
N T
D16s512
101
N T
89
N T
D16s398
114
N T
D16s402
111
N T
104
N T
D16s422
91
N T
58
N T
D16s516
106
N T
D16s504
59
N T

I.d Discussion

DCIS was always suspected as the most probable precursor lesion to invasive carcinoma (2). Dupont and Page found a greatly increased risk of subsequent invasive breast cancer in women with history of DCIS positive biopsy. Recently several laboratories has provided molecular evidence that further substantiates the model of progression from DCIS to invasive breast cancer (5, 13-15). Nevertheless we still know very little on the role of specific genetic abnormalities at preinvasive stages of breast cancer development. We have recently demonstrated that specific chromosome arms are more frequently affected by allelic losses at the DCIS stage (5). Based on these results, we postulated that these genetic alterations in DCIS are early events and may play an important role in the genesis of invasive breast cancer. In that study we observed that marker D16S413 located on the telomeric band 16q24.3 was significantly affected by allelic losses when compared with other loci. This prompted us to extend our analysis on the q arm of chromosome 16 generating a high resolution allelotype of this chromosome arm. Chromosome 16q has been suggested as a site for the occurrence of primary cytogenetic structural abnormalities in the development of breast cancer (17,18). In particular 16q was shown to systematically participate in nonrandom translocations with chromosome 1; and 16q deletions were also frequently observed (17,18). Furthermore breast cancer allelotypic studies have systematically shown the common occurrence of allelic losses affecting the chromosome 16q arm. In addition to our observations (5) other investigators have also reported the occurrence of frequent allelic losses affecting chromosome 16q in DCIS (7,16).

It has been suggested that probably more than one putative tumor suppressor loci of interest in breast cancer may reside in 16q. At least two regions of chromosome 16q has been previously reported consistently for the occurrence of LOH: 16q21 and 16q24.2-qter (7,8,19). Here we report that 31 of 35 DCIS tumors (89%) showed allelic losses in one or more loci of the long arm of chromosome 16. This is a very high incidence, compared to approximately 50% or less LOH in other studies of invasive breast carcinomas (7,19). We explain this difference in incidence by the use of tissue microdissection in our study which improved the separation of tumor DNA from normal stromal contaminants. This is particularly important when dealing with small islands of tumor cells such as in DCIS. In addition, in our study we used highly polymorphic microsatellite markers which provide a high number of informative samples.

In our analysis of DCIS lesions we identified three distinct regions with a very high percentage (~70% or above) of allelic losses among informative DCIS samples. Two of them agree with previously described areas: 16q21 at locus D16S400 and 16q24.2 at locus D16S402. However, the region with the highest incidence of LOH observed in our study spans between markers D16S515 to D16S516 (see Table 1). Within this region the D16S518 locus was the most frequently affected, since 20 of 26 DCIS tumors (77%) showed LOH at this locus.

These observations strongly suggest that a putative tumor suppressor gene/s may possibly be harbored at or in the vicinity to this locus. Furthermore, the incidence of allelic loss at D16S518 could potentially be higher since some few tumors that preserve heterozygosity at this locus showed losses in flanking markers (e.g. tumors 58 and 102).

This could be due to homozygous deletions affecting this region which are very difficult to judge because of the nature of the PCR mediated approach used.

Based on a YAC contig spanning the region of interest we can approximately estimate that the minimum region with the highest frequency of LOH appears no larger than 2-3Mb in size (Figure 4). Furthermore based on the cytogenetic location of markers D16S504 and D16S516 and the distance to D16S518 this area should be contained within bands 16q23.3-q24.1. This region appears different from another area of frequent LOH more distally located at locus D16S402 in band 16q24.2. We substantiate this observation within the fact that both areas are 17 cM apart based on the Genethon Linkage Map (March 1996) and several Mb away based on comprehensive chromosome 16 physical and genetic Map (8).

It will be particularly important to analyze for the occurrence of allelic losses the chromosome regions identified in this report in other less advanced hyperplastic breast lesions. This analysis will be useful in our understanding of breast carcinogenesis and may help in the identification of markers with diagnostic-prognostic significance.

I.e Conclusions

Allelic losses or imbalances affecting chromosome arm 16q appeared to be early abnormalities since they were observed in a significant number of breast DCIS lesions in our previous study (5). In order to define the minimum region of LOH we performed a high resolution allelotype of 35 DCIS cases and completed a deletion map of chromosome 16q by means of paraffin tissue microdissection and PCR microsatellite analysis of 22 markers. We observed an striking high frequency of LOH in 16q, with 31 of 35 tumors (89%) affected. We identified three distinctive areas with high LOH. Two areas were previously described and correspond to 16q21 and 1624.2-qter. The third one and most commonly affected area spans from markers D16S515 to D16S516, and the most affected locus was at D16S518 in which LOH was observed in 20 of 26 informative cases (77%), we estimate its location at sub-region q23.3-q24.1. The region of highest LOH spans approximately 2-3 Mb in size, as determined by a YAC contig. Such a high frequency of LOH at a preinvasive stage of breast cancer suggests that a candidate tumor suppressor gene/s at this location may play an important role in breast carcinogenesis. Further studies are necessary in order to identify the gene/s of interest.

I.f References

1. Devilee, P., and Cornelisse, C.J. Somatic genetic changes in human breast cancer. Biochim. Biophys. Acta, 1198:113-130, 1994.
2. Dupont, W.D. and Page, D.L. New Engl. J. Med. 312:146-151, 1985
3. Cavenee, W.K., Koufos, A., and Hansen, M.F. Recessive mutant genes predisposing to human cancer. Mut. Res., 168:3-14, 1986.
4. Chen, T., Dhingra, K., Sahin, A., et al. Technical approach for the study of the genetic evolution of breast cancer from paraffin-embedded tissue sections. Breast Cancer Res. Treat., in press, 1995.
5. Aldaz, C.M., Chen, T., Sahin, A., Cunningham, J., and Bondy, M. Comparative allelotype of *in situ* and invasive human breast cancer: high frequency of

microsatellite instability in lobular breast carcinomas. Cancer Res., 55:3976-3981,1995.
6. Lindblom, A., Rotstein, S., Skoog, L., Nordenskjold, M. and Larson, C. Deletions onb chromosome 16 in primary familial breast carcinomas are associated with development of distant metastases. Cancer Res., 53:3703-3711, 1993.
7. Tsudaa, H., Callen, D.F., Fukuutomii, T., Nakkamura, Y. and Hirohashi, S. Allele loss on chromosome 16q24..2-qter occurs frequently in breast cancer irrespectively of differences in phenotype and extent of spread. Cancer Tes., 54:513-517, 1994.
8. Cleton-Jansen, A.M., Moerland, E.W., Kuipers-Dijkshoorn, N.J., Callen, D.F., Sutherland, G.R., Hansen, B., Devilee, P. and Cornelisse, C.J. At least two different regions are involved in allelic imbalance on chromosome arm 16q in breast cancer. Genes, Chromos. & Cancer, 9:101-107, 1994.
9. Carter, B.S., Ewing, C.M., Ward, W.S., Treiger, B.F., Aalders, T.W., Schalken, J.K., Epstein, J.I., and Isaacs, W.B. Allelic loss of chromosomes 16q and 10q in human prostate cancer. Proc. Natl. Acad. Sci. USA, 87:8751-8755,1990.
10. Nishida, N., Fukuda, H., Sadamoto, T., Isowa, G., Honda, K., Yamaoka, Y., Ikenaga, M., Imura,H., and Ishizaki, K. Accumulation of allelic loss on arms of chromosomes 13q, 16q, and 17p in the advanced stages of human hepatocellular carcinoma. Int. J. Cancer, 51:862-868, 1992.
11. Maw, M.A., Grundy, P.E., Millow, L.J., Eccles, M.R., Dunn, R.S., Smith, P.J., Feinberg, A.P., Law, D.J., Paterson, M.C., Telzerow, P.E., Callen, D.F., Thompson, A.D., Richards, R.I. and Reeve, A.E. A third wilms'-tumor locus on chromosome 16q. Cancer Res., 52:3094-3098, 1992.
12. Hudson, T.J., Stein, L.D., et al., An STS-based map of the human genome. Science 270:1945-1954, 1995.
13. Zhuang, Z., Merino, M.J., Chuaqui, R., Liotta, L., and Emmert-Buck, M.R. Identical allelic loss on chromosome 11q13 in microdissected *in situ* and invasive breast cancer. Cancer Res., 55:467-471, 1995.
14. O'Connell, P., Pekkel, V., Fuqua, S., Osborne, C.K., and Allred, D.C. Molecular genetics studies of early breast cancer evolution. Breast Cancer Res. Treat., 32:5-12, 1994.
15. Radford, D.M., Phillips, N.J., Fair, K.L., Ritter, J.H., Holt, M., and Donis-Keller, H. Allelic loss and the progression of breast cancer. Cancer Res., 55:5180-5183, 1995.
16. Radford, D.M., Fair, K.L., Phillips, N.J., Ritter, J.H., Steinbrueck, T., Holt, M.S., and Donis-Keller, H. Allelotypiing of ductal carcinoma in situ of the breast: deletion of Loci on 8p, 13q, 16q, 17p and 17q. Cancer Res., 55:3399-3405, 1995.
17. Dutrillaux, B., Gerbault-Seureau, M., and Zafrani, B. Characterization of chromosomal anomalies in human breast cancer. A comparison of 30 paradiploid cases with few chromosome changes. Cancer Genet. Cytogenet., 49:203-217, 1990.
18. Pandis, N., Heim, S., Bardi, G., Idvall, I., Mandahl, N., and Mitelman, N. Whole-arm t(1;16) and i(1q) as sole anomalies identify gain of 1q as a primary chromosomal abnormality in breast cancer. Genes Chromosomes & Cancer, 5:235-238, 1992.
19. Sato, T., Tanigami, A., Yamakawa, D., Akiyama, F., Kasumi, F., Sakamoto, G., and Nakamura, Y. Allelotype of breast cancer: cumulative allele losses promote tumor progression in primary breast cancer. Cancer Res., 50:7184-7189, 1990.

II. Subtitle: Replication error phenotype in breast cancer

II.a Introduction

In our original allelotyping studies (1) we observed that numerous breast cancer samples, in particular of the invasive lobular subtype, showed frequent abnormalities in the allele size migration in polyacrylamide gels when compared with the matched normal controls. Abnormalities in size of simple sequence nucleotide repeats is a phenomenon described as microsatellite instability (2). This phenomenon has been described as a characteristic of tumors from patients carrying the autosomal dominant predisposition to tumors of the colon and endometrium, known as hereditary nonpolyposis colon cancer (2). These studies led to the identification of a group of human DNA mismatch repair genes as the cause of such general genomic instability phenomenon. Germline mutations in either the Escherichia *coli mutS* homolog *hMSH2* or the *mutL* homologs *hMlH1*, *hPMS1* and *hPMS2* have been found in different subsets of hereditary nonpolyposis colon cancer kindreds (3,4). Microsatellite instability, also known as replication error phenotype, has also been reported to occur at various frequencies in various sporadic neoplasias other than colon cancer, such as cancers of the endometrium (5), stomach (6), esophagus (7), bladder (8) and other tissues. Yee et al. (9) reported microsatellite instability in 20% of breast cancers. Recently, Glebov et al. (10) observed that individuals with a family history of breast cancer and with *p53* mutations had a higher frequency of abnormalities of chromosome 17 loci.

In our study (1) of unselected breast cancer cases and mostly dinucleotide repeat markers, we observed the replication error-positive phenotype (RER+) in 16 of the 75 breast cancer samples (21%). This figure is similar to that reported by Yee et al. (9). Interestingly however, when analyzed by histological subtype, only 13% (7 of 52 tumors) of ductal tumors (DCIS plus invasive ductal tumors) showed the RER^+ phenotype, in contrast with 39% (9 of 23) of infiltrating lobular breast carcinomas.

Our data suggest that invasive lobular breast carcinomas appear to arise by a mechanism of carcinogenesis different from that of ductal breast carcinomas and may constitute a possible different pathologic entity. These findings also support previous observations of different clinical behaviors of lobular breast tumors and ductal tumors (11-13). The diagnosis of lobular breast carcinoma has been associated with a higher risk for development of multifocal or subsequent contralateral breast cancer (11,12). The possibility exists that some patients that develop lobular breast tumors could harbor or develop mutations in any of the DNA mismatch repairs genes in the mammary epithelium, thus producing a field defect and constituting a facilitating event for the development of secondary mutations leading to tumor development.

During this past year we extended those earlier observations (5) by analyzing a set of RER+ breast cancer samples for mutations in the DNA mismatch repair gene HMSH2 (manuscript in preparation). As earlier indicated this is the most commonly mutated gene in HNPCC kindred.

II.b Materials and Methods

Paraffin embedded tissue sections were obtained from a set of nine breast cancer samples positive for microsatellite instability at multiple loci (more than 5 independent loci).
Matching normal control tissues were also obtained from each patient.
DNA samples were obtained as previously described (14).
Oligonucleotide primers (flanking and nested) for analysis of the 16 exons were synthesized according to the method described by Kolodner et al. (15).
PCR conditions and cycle sequencing were performed according to the same authors (15)

II.c Results

The sequence analysis of the 16 exons of the HMSH2 gene did not show evidence for germinal or somatic mutations. The HMSH2 sequence obtained in all nine RER+ breast cancer cases was normal.

II.d Conclusions

The phenomenon of microsatellite instability observed in the sporadic breast cancer cases studied appears to be independent of mutations in the prototype and most frequently mutated DNA mismatch repair gene HMSH2. Our results are comparable to studies in RER+ sporadic colon cancer cases (16). Although other DNA mismatch repair genes have not been analyzed these data suggests microsatellite instability in sporadic breast cancer may be the result of a different mechanism and genes to that described in the HNPCC syndrome.

II.e References

1. Aldaz, C.M., Chen, T., Sahin, A., Cunningham, J., and Bondy, M. Comparative allelotype of *in situ* and invasive human breast cancer: high frequency of microsatellite instability in lobular breast carcinomas. Cancer Res., 55:3976-3981,1995.
2. Aaltonen, L.A., Peltomäki, P., Leach, F.S., Sistonen, P., Pylkkänen, L., Mecklin, J.-P., Järvinen, H., Powell, S.M., Jen, J., Hamilton, S.R., Petersen, G.M., Kinzler, K. W., Vogelstein, B., and de la Chapelle, A. Clues to the pathogenesis of familial colorectal cancer. Science, *260*: 812-816, 1993.
3. Fishel, R., Lescoe, M.K., Rao, M.R.S., Copeland, N.G., Jenkins, N.A., Garber, J., Kane, M., and Kolodner, R. The human mutator gene homolog *MSH2* and its association with hereditary nonpolyposis colon cancer. Cell, *75*: 1027-1038, 1993.
4. Bronner, C.E., Baker, S.M., Morrison, P.T., Warren, G., Smith, L.G., Lescoe, M.K., Kane, M., Earabino, C., Lipford, J., Lindblom, A., Tannergard, P., Bollag, R.J., Godwin, A.R., Ward, D.C., Nordenskjold, M., Fishel, R., Kolodner, R., and Liskay, R.M. Mutation in the DNA mismatch repair gene homologue *hMLH1* is associated with hereditary non-polyposis colon cancer. Nature, *368*: 258-261, 1994.
5. Risinger, J.I., Berchuck, A., Kohler, M.F., Watson, P., Lynch, H.T., and Boyd, J. Genetic instability of microsatellites in endometrial carcinoma. Cancer Res., *53*: 5100-5103, 1993.

6. Han, H.-J., Yanagisawa, A., Kato, Y., Park, J.-G., and Nakamura, Y. Genetic instability in pancreatic cancer and poorly differentiated type of gastric cancer. Cancer Res., *53*: 5087-5089, 1993.
7. Meltzer, S.J., Yin, J., Manin, B., Rhyu, M.-G., Cottrell, J., Hudson, E., Redd, J.L., Krasna, M.J., Abraham, J. M., and Reid, B. J., Microsatellite instability occurs frequently and in both diploid and aneuploid cell populations of Barrett's-associated esophageal adenocarcinomas. Cancer Res., *54*: 3379-3382, 1994.
8. Gonzalez-Zulueta, M., Ruppert, J.M., Tokino, K., Tsai, Y.C., Spruck III, C.H., Miyao, N., Nichols, P.W., Hermann, G.G., Horn, T., Steven, K., Summerhayes, I.C., Sidransky, D., and Jones, P.A. Microsatellite instability in bladder cancer. Cancer Res. *53*:5620-5623, 1993.
9. Yee, C.J., Roodi, N., Verrier, C.S., and Parl, F.F. Microsatellite instability and loss of heterozygosity in breast cancer. Cancer Res., *54*: 1641-1644, 1994.
10. Glebov, O.K., McKenzie, K.E., White, C.A., and Sukumar, S. Frequent *p53* gene mutations and novel alleles in familial breast cancer. Cancer Res., *54*: 3703-3709, 1994.
11. Tavassoli, F.A. In: Pathology of the Breast, Appleton & Lange Norwalk, Connecticut, 1992.
12. Silverstein, M.J., Lewinsky, B.S., Waisman, J.R., Gierson, E.D., Colburn, W.J., Senofsky, G.M., and Gamagami, P. Infiltrating lobular carcinoma. Is it different from infiltrating duct carcinoma? Cancer, *73*: 1673-1677, 1994.
13. Harris, M., Howell, A., Chrissohou, M., Swindell, R.I.C., Hudson, M., Sellwood, R.A. A comparison of the metastatic pattern of infiltrating lobular carcinoma and infiltrating duct carcinoma of the breast. Br. J. Cancer *50*:23-30, 1984.
14. Chen, T., Dhingra, K., Sahin, A., et al. Technical approach for the study of the genetic evolution of breast cancer from paraffin-embedded tissue sections. Breast Cancer Res. Treat., in press, 1995.
15. Kolodner, R.D., Hall, N.R., Lipford, J., Kane, M.F., Rao, M.R.S., Morrison, P., Wirth, L., Finan, P.J., Burn, J., Chapman, P., Earabino, C., Merchant, E., and Bishop, D.T. Structure of the human MSH2 locus and analysis of two Muir-Torre kindreds for msh2 mutations. Genomics, 24:516-526, 1994.
16. Liu, B., Nicolaides, N.C., Markowitz, S., Willson, J.K.V., Parsons, R.E., Jen, J., Papadopolous, N., Peltomäki, P., Chapelle, A., Hamilton, S.R. Kinzler, K.W., and Vogelstein, B. Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. Nature Genetics, 9:48-55, 1995.